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A METHOD FOR THE STUDY OF THE WATER CURRENTS OF INVERTEBRATE CILIARY FILTER FEEDERS

by
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The problem of measuring the pumping rate of a ciliary filter-feeding organism has no completely adequate solution at present. The best methods so far developed are limited to a few specialized groups of filter-feeders, and no method offers a precise determination of absolute instantaneous rate of flow.

The study of flow rates can be pursued by two broad categories of methods: those that do and those that do not require contact between apparatus and animal. Bidder (1923) describes a simple method of the second type for measuring the velocity of the excurrent jet of an osculate sponge. A carmine suspension is delivered to the ostia by a hand pipette. Then, in Bidder's words:

> I found the coloured jet marked by dark beads or nodes, caused by my pulse shaking the pipette; the length between any two nodes, divided by three-quarters of a second, gives the core-velocity at that part of the jet.

The analysis of various sponge currents by this method led to an empirical formula:

$$L = (12 \pm 2)\ VB\ (1 - 0.023[20 - t])$$
cm. sec. °C.

L = Length of jet. B = diameter of jet at osculum. V = Velocity of jet. t = temperature.

The value of Bidder's work is reduced by a single unfortunate circumstance. In accord with the style of many papers of that time, he reports conclusions primarily, and includes little of the supporting data. Although he spent six years at the Naples Zoological Station and observed over a thousand individuals, his calculations are illustrated by figures drawn from a single sponge (number A-11). Jørgensen (1955) calls Bidder's paper the only reliable determination of the work done by the ciliary pumping mechanism of a filter-feeder. Yet the range of variation and the mathematical reliability of Bidder's results cannot be obtained from his report.

Other methods involving no physical contact with the organism are the particle removal method and the vane method. The particle removal method dates from Dodgson (1928) who determined the rate of clearing of mud suspensions. Jørgensen (1949b) further developed the method of Fox, Sverdrup, and Cunningham (1937) using dyes, graphite suspensions, and algae. Chipman and Hopkins (1954) determined the rate of clearance of radioactive algal suspensions by Pecten. Their work represents the best short term measurement made by the particle removal method.

Regardless of the material used or the methods of determining its concentration, there are several problems associated with this technique. MacGinitie (1941) and Jorgensen (1949a) have demonstrated relationships between particle concentration and feeding rate. It appears probable that all filter-feeders require some method of sensing particle density, and that feeding rates and methods are affected by the number and kinds of particles in the water. If the animal can sense the change in particle concentration used to measure its pumping rate, it may alter that rate during the course of the experiment to match each new particle density. The problems encountered in creating stable, non-toxic, monodisperse suspensions of known particle size complicate the comparison of separate experiments.

The vane method of Hopkins (1933) uses a conical cup suspended in the excurrent stream. Deflections of the cup caused by variations in the stream are registered on a kymograph. Hopkins points out that this method is limited to the comparison of flow rates and is incapable of determining absolute amounts.

Of the various techniques which involve apparatus directly attached to the animal, the apron technique is probably the least disturbing to normal behaviour. The use of an apron, an impermeable membrane separating the incurrent and excurrent openings, was originally suggested by Moore (1910). The separation of the two openings permits water pumped through the animal to be isolated, collected, and measured. The apparatus was improved by Loosanoff, Engle, Galtsoff, and other workers. The most elaborate apron measuring device is found in Loosanoff and Engle (1947). The apron is limited in application to shelled forms whose incurrent and excurrent orifices are widely and distinctly separated. The associated equipment is elaborate and cumbersome, and while in theory it produces no pressure differential, in practice overflow back pressure cannot be reduced to zero.

Most filter-feeding organisms offer the possibility of isolating either the incurrent or excurrent flow in a tube attached directly to the animal. The rates of flow of such isolated streams have been determined by a variety of methods. The technique of Wells and Dales (1951), developed for polychaete tube dwellers, has been applied to a ciliary filter-feeder by Hoyle (1953). This method depends on the creation of a back pressure. A continuous record can be obtained, but the resistance of the back pressure may account for the rather low values of Hoyle's measurements. Hoyle includes in his paper a criticism of Hecht (1916), who measured the speed of carmine particles fed through a glass incurrent cannula. Hoyle's own drop migration method (1953) and Galtsoff's carmine method (1926) share certain difficulties with the method of Hecht. Any cannulation procedure involves continuous contact stimulation of the animal, and the restricted bore of the cannula creates a viscous resistance not experienced in nature. Galtsoff has introduced several other cannulation methods, all of which involve severe handling of the animal. His usual procedure is to prop apart the shells of an oyster with a glass rod and to pack cotton around the edges of an inserted tube. Leaks are detected with carmine, but disturbances of the animal are not measurable.

It would be desirable to find a method of measuring instantaneous flow rate in ciliary filter-feeders which did not depend on handling the animal or on modifying its environment beyond the limits found in nature.

This paper presents an attempt to develop such a method. The work done is in a large part the product of the patient encouragement and criticism of Dr. Ralph I. Smith, and of many discussions with other members of the Zoology Department of the University of California at Berkeley, in particular, Professor Jonas Gullberg and Gordon W. Ellis.

Qualitative Observations Using the Streaming Birefringence of Tobacco Mosaic Virus

When large molecules with a high degree of axial asymmetry are aligned in solution they exhibit anisotropy. Molecular alignment can be created by movements of the solution. The degree of anisotropy is a function of the size of the particles, their shape, and concentration, and the rate of flow of the stream of solution. This effect, called streaming birefringence, can be used to demonstrate currents in a liquid.

A solution of 5 gram/liter of Tobacco Mosaic Virus (the TMV used was a supernatant from the centrifugal purification of infective virus, supplied through the courtesy of Dr. C. A. Knight of the Biochemistry and Virus Laboratory, University of California, Berkeley) in sea water gives a usable visualization of the excurrent streams of small *Mya arenaria* (L.). A 5 x 5 x 20 cm. aquarium filled with the solution was placed between two crossed sheets of Polaroid HN32 linear polarizer. On one side of the aquarium a Leica 35 mm. camera, stopped to f4, was focussed on the test animal. On the other side a pair of lights was set up behind a ground glass screen. One bulb was red filtered and provided the normal viewing light. The other was a Braun "Hobby" photographic flash which gave enough light to expose Kodak Tri-X film for optimum development in D76. A clam, relaxed and pumping in the tank, created a birefringent area extending from the excurrent opening. Several attempts were made to measure the rate of this excurrent stream. The only method that met with even partial success was an attempt to emulate Bidder by mechanically injecting drops of virus-free sea water into the moving stream. It was hoped that the drops would stand out as black areas in the light stream, since they would lack the polarizing properties of the surrounding medium. The normal ciliary-propelled stream of *Mya* is smooth and laminar, easily distinguishable from the turbulent jet created by a muscular contraction of the body wall. Such a laminar stream slows down by picking up the surrounding water and accelerating it in the direction of the primary current. The birefringence of this secondary current is sufficient to mask all but the densest black spaces in the main stream. Drops sufficiently large to mark the stream were diffuse and hard to measure.

Although streaming birefringence did not prove practical as a quantitative tool, its advantages as a qualitative method of observation should not be ignored. The morphology of the excurrent stream, its laminar character, slow widening, and rapid decay are easily visible. The Tobacco Mosaic Virus is apparently not irritating to the clam. Frequent filtration, occassional centrifugal purification, and a few milliliters of penicillin-streptomycin solution will preserve a half liter of the dilute virus for several months. In all of the experiments apparatus and animals were maintained at 15°C.

Photographic Determination of Water Movement

A multiple photographic exposure of a scene in which some objects move and others are motionless will permit measurement of the existing rates of motion. By fixing the time intervals between individual exposures the time required for a particle to move from one position to the next can be determined, and the distance moved can be calculated from the distance recorded on the photographic negative.

The camera used in the previous experiments was modified to permit multiple exposures by the addition of a second rotating shutter in front of the lens. The shutter was a six inch disk, driven by a geared synchronous motor at 66 rpm, with segments cut from its edge to generate the desired exposure sequence. The camera's own focal plane shutter was held open for one or more revolutions of the multiple shutter, and the film recorded a series of exposures whose time relationships were accurately known.

A thin aquarium (7 x 25 x 40 cm.) was filled with a suspension of aluminum dust in sea water. The moving dust grains could be photographed by the modified camera as rows of dots. The dust is "Albron" powder, a standard paint pigment sold by Alcoa. Suspensions made by shaking a few grams of powder in a small volume of sea water, and diluting to the required concentration, are stable for several hours. Individual particles range from 1/10 to 1/100 mm. in greatest dimension, but the thickness of the sheetlike fragments is on the order of 1/1000 mm. Particle concentrations as low as twenty per cubic centimeter can be used. Such suspensions appear perfectly clear unless illuminated by an intense beam of parallel light which reveals the aluminum grains as smoke particles are revealed by a shaft of sunlight.

The aquarium was lighted from above at right angles to the line of sight of the camera. A microscope lamp with a 6 cm. lens, focussed to a parallel beam, was intense enought to allow the light reflected by the particles to be photographed. To increase the visibility of the particles, the aquarium was backed with dull black paper.

A size scale was made by blackening alternate squares of a one inch square of one tenth inch ruled graph paper. In use the scale was placed on the wall of the aquarium behind the experimental animal. The checkerboard pattern was used to allow the scale to be reconstructed from a slightly out-of-focus view. Since the animal was nineteen inches from the camera, the maximum apparent reduction of the size scale was 5%, or about equal to the error created by estimating the boundaries of squares in the photograph.

An animal under test was positioned so that both siphon openings presented clear profiles to the camera. The areas of the circular openings were calculated from their measured diameters. A single picture taken under these conditions gives a velocity diagram of the water in the tank. Convection currents must be avoided, since they warp the paths of the particles from the pattern created by the animal.

If we assume the volume of the animal to be constant during the time of exposure, the product of incurrent velocity and the area of the incurrent siphon must be equal to the product of excurrent velocity and area. The two streams behave in different fashions, and the differences must be understood before the velocities at points distant from the animal can be extrapolated to the siphon tip.

The incurrent water moves in a very simple pattern. Consider an isolated incurrent siphon. Near the siphon opening a small volume of water moves to enter the animal. A shell of water surrounding this volume moves in to occupy the space left by its removal, another shell moves in to occupy the new vacancy, and so on outward. It is obvious that the shells will enlarge their areas and decrease their thicknesses with increasing distance from the siphon tip, since the volume of the shells is constant and each must inclose its predecessor. In fact, the shell thickness, which is equivalent to the linear rate of water movement, will vary inversely as the square of the distance from the siphon tip.

The excurrent water disturbs this regular motion, since, as was seen in the Tobacco Mosaic Virus studies, it loses energy by accelerating the surrounding water. The inner portion of the excurrent stream remains at a nearly constant speed while the outer boundary is slowed by the surrounding incurrent water. The incurrent flow is actually reversed by contact with the outflow. Bidder's formula will not apply to such a situation, since the outgoing jet is opposed, not by still water, but by an actively moving stream.

Measurements of flow rate must be taken either at the center of the excurrent stream or at a considerable distance from it, in order to avoid the anomalous area where the two currents touch.

Reduced weight was determined by weighing the animal in a glass graduate filled to a constant level. Such a measurement offers a quick and repoducible way to characterize animals with a variable internal water space.

Ciliary flow in Mya shows occasional discontinuities of a regular form. The clam first opens its incurrent siphon and begins to create a current. This priming current is probably established by a relaxation of the adductors and a consequent increase in internal volume. Starting the flow in this way would relieve the cilia of the heavy burden of the initial accelerative load. After the incurrent flow is established, the excurrent siphon opens and begins to emit its jet. The excurrent jet rises to a maximum rate of flow and maintains activity for minutes or hours at a time. It is not possible to isolate an animal from low frequency vibration in the building where this work was carried out. There is no evidence, therefore, of spontaneous rhythmicity in the time devoted to ciliary pumping. Either the excurrent flow, the incurrent flow, or both may stop abruptly, or the entire cycle may be interrupted by a muscular expulsion of water from one or both jets. Either cessation of flow or squirting may be induced by a disturbance such as a door slamming, an engine starting, or a sudden changing of illumination.

Although Platyodon has a much greater incurrent to excurrent area ratio than Mya, its pattern of ciliary currents is very similar. The muscular pumping is rather different and may reflect the needs of an animal that is unable to withdraw its siphon and relocate it inches away. The excurrent siphon of Platyodon is equipped with a translucent collar which functions as a valve. When placed in a heavy suspension of particles, Platyodon tends to expel sudden jets of water. After each jet the body expands, but the valve prevents water from re-entering the excurrent siphon. This arrangement may serve to allow the clam to increase the amount of water passing through its gills without altering the direction of flow.

A recent summary of filtering rate determinations is included in Ballantine and Morton (1956). Ranges of from 3.9 to 20 liters/hour are recorded for Ostrea virginica of a size comparable with that of the Platyodon cancellatus (Conrad) used in the present study. The animals are not strictly comparable, and the rate of 0.5 liters per hour determined for Platyodon can only be said to be of approximately the correct order of magnitude. The Mya values can be compared with the published values for Mytilus with the same reservation. Mytilus exhibits a range of from 1.1 to 1.9 liters/hour as compared with the present measurement of 0.25 liters/hour for Mya arenaria (Linn.).

The aluminum dust-photographic method of studying ciliary currents can be applied to any filter-feeder. Those organisms which possess irregular siphon openings may be analyzed by determining the area of the openings planimetrically from auxiliary photographs. The particle concentrations used compare favorably with the lowest particle densities found in nature. The animal is subjected to constant light and temperature conditions, no machinery is attached to the animal, and no resistance is offered to normal movement. The only specialized piece of apparatus, the rotating shutter, is easily constructed from inexpensive and readily available parts.

This technique allows an exact and simple determination of absolute instantaneous flow rates, and in addition permits the quantitative or qualitative study of flow patterns, their interaction with other currents, and their variation under environmental changes.

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COLOR PHASES IN MONADENIA FIDELIS (GRAY)

by
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(Plate 18)

While making a comparative study of the populations of the large land snails inhabiting the Klamath Mountains in extreme northern California, some rather interesting distributional patterns were noted. The project chiefly concerned the distribution and speciation in the genus Monadenia Pilsbry, and as a matter of course special attention was given to coloration and color pattern. Usually, it was noted that a species or race inhabited a specific drainage area; from the glacial cirques that headed such an area, to the downstream junction with some major watercourse. Therefore it afforded some interest when a rather distinctive sequence of color phases was found that